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Mitochondrial DNA and plumage evolution in the white wagtail *Motacilla alba*

Alexandra Pavlova, Robert M. Zink, Sievert Rohwer, Evgeniy A. Koblik, Yaroslav A. Red'kin, Igor V. Fadeev and Evgeniy V. Nesterov

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We analyzed sequences of two mitochondrial DNA (mtDNA) gene regions (control region and ND2) totaling 1477 base-pairs from 232 specimens of the white wagtail *Motacilla alba* obtained from 27 localities throughout Eurasia. Although overall haplotype diversity was relatively low (0.79) and the most common haplotype was shared by 45% of individuals, belonging to six subspecies, a high level of population differentiation was detected. The mtDNA tree revealed three clades: (1) most individuals from Krasnodar (belonging to *M. a. alba* subspecies), (2) all individuals from Almaty and some from Primor'e (belonging to *M. a. personata*, *M. a. lugens* and *M. a. leucopsis* subspecies), and (3) the remaining individuals (representing all subspecies and all localities except Almaty). We suggest that these three clades represent historically isolated populations that relatively recently came into secondary contact in Krasnodar and Primor'e. None of the six subspecies were reciprocally monophyletic in the mtDNA tree. The Krasnodar population appeared to receive immigrants from other localities, but distinctive haplotypes from this locality did not appear elsewhere, suggesting asymmetric gene flow. Signatures of recent gene flow between northern populations were detected, and there was no evidence of isolation by distance within the northern group of populations. Mismatch distributions for most localities were consistent with population expansions. We also analyzed 12 male plumage characters from 93 study skins sampled from 24 populations. Phylogenetic trees resulting from separate genetic and morphological analyses were incongruent. Plumage evolution seems to be under strong sexual or natural selection, which favors particular phenotypes in various areas irrespective of the mitochondrial background. Dispersal events at different evolutionary times could have obscured the effects of earlier isolation events. The mtDNA data does not support species status for *M. a. lugens* and *M. a. personata*, which shared haplotypes with other subspecies of *M. alba*. We recommend that *M. lugens* and *M. personata* are placed as junior synonyms of *M. alba*.

A. Pavlova and R. M. Zink (correspondence), Bell Museum of Natural History, University of Minnesota, St. Paul, MN 55108, E-mail: rzink@biosci.umn.edu. S. Rohwer, Burke Museum and Department of Zoology, University of Washington, Seattle, Washington 98195-3010. E. A. Koblik and Y. A. Red'kin, Moscow State University, Zoological Museum, Moscow, Bol'shaya Nikitskaya 6, Russia. I. V. Fadeev and E. V. Nesterov, State Darwin Museum, 57 Vavilova street, Moscow 117292, Russia.

The white wagtail *Motacilla alba* is a common passerine bird, whose range covers most of Eurasia, from the British Isles to Chukotka and western Alaska, and from the coast of the Arctic ocean to roughly 36° N latitude in Europe, and to 20° in southeastern Asia (Dement'ev and Gladkov 1954, Cramp 1988, Badyaev et al. 1996). White wagtails occur in a variety of habitats, excluding tall and dense vegetation, from cold regions in high latitudes to arid, hot climates, and are often associated with human settlements (Cramp 1988). Geographic variation of the

white wagtail is largely defined by the distribution of black, white and gray in different regions of the body (Stepanyan 2003). Separating the white wagtail into subspecies has been difficult because different forms may hybridize where their ranges meet (Dement'ev and Gladkov 1954, Stepanyan 2003), and because individuals in some subspecies (like *M. a. persica*) resemble neighboring races (Cramp 1988). Sexual dimorphism exists in some geographical races, which along with the gradual geographic transitions from one form to another, further complicates assigning subspecies status to individuals.

Cramp (1988) distinguishes four subspecies-groups based on similarity of head pattern of breeding males: (1) *M. a. personata* and *M. a. alboides*, (2) *M. a. ocularis* and *M. a. lugens*, (3) *M. a. alba* (including *M. a. dukhunensis*) and *M. a. yarrellii*, and (4) *M. a. baicalensis* and *M. a. leucopsis*. The first member of each subspecies pair has a gray back and upperparts which differentiates it from the latter member of the pair, which has a black back and upperparts. Further, Cramp (1988) suggests that group 1 and group 2 are related, and that group 3 and group 4 are related, but that groups 1–2 were more "distant" from each other than were groups 3–4. The black-backed wagtail *M. a. lugens*, which inhabits coastal habitats of Kamchatka, Sakhalin and Primor'e, has recently colonized Alaska; it was given species status by the American Ornithologists' Union (1983). Stepanyan (2003) recognizes *M. a. lugens* and *M. a. personata* as species, whereas Alström and Mild (2003) use *M. lugens* and *M. personata* as synonyms of *M. alba*.

Ödeen and Alström (2001) assessed the genetic relationships among different subspecies of *M. alba* by comparing mitochondrial (mt) and nuclear DNA sequences. Their nuclear tree showed no resolution of subspecies, moreover one of two sampled individuals of *M. grandis* was found in *M. alba* clade. On the basis of the mtDNA, Ödeen and Alström (2001) recognized two groups: (1) the *alboides* group, which included the subspecies *M. a. alboides*, *M. a. leucopsis* and *M. a. personata*, and (2) the *alba* group, which included *M. a. alba, M. a. yarrellii, M. a. baicalensis, M. a. ocularis, M. a. lugens,* and *M. a. subpersonata*. The breeding sites of some of the sampled birds used in Ödeen and Alström's (2001) mtDNA study were unknown, because blood from migrating individuals was used. Ödeen and Alström (2001) assumed that subspecies were monophyletic, but were unable to test this assumption because for most subspecies a single individual was used. In the case of *M. a. subpersonata*, the two individuals they sampled were not each other's closest relatives. Zink et al. (2003) showed that incorporating multiple individuals from known breeding localities often dissolves clades thought to support subspecies. Furthermore, phenotypic similarities between subspecies may be caused by convergent evolution due to natural or sexual selection (Pavlova et al. 2003).

Extensive geographic sampling with multiple individuals taken from the same locality is needed to document the mtDNA pattern of variation, to test subspecies limits, and to clarify the evolution of plumage patterns. In our study we made no a priori assumptions about monophyly and used geographic samples of breeding individuals, rather then currently recognized subspecies. We characterize the geographic distribution of variation in plumage phenotypes and mtDNA haplotypes for White wagtails collected across Eurasia during the breeding season.

Material and methods

Sampling

A total of 232 white wagtails from 27 populations sampled across Eurasia was collected during breeding season (Fig. 1). From almost all specimens a study skin with spread wing was preserved and deposited at the Burke Museum, University of Washington, Seattle, the Moscow State University Zoological Museum, Moscow, Russia, State Darwin Museum, Moscow, Russia, and the Bell Museum, University of Minnesota, St. Paul. Tissue samples were either preserved in 96% ethanol, frozen in liquid nitrogen, or stored in lysis buffer (Longmire et al. 1997). The white-browed wagtail *M. maderaspatensis*, endemic to the Indian subcontinent, was used as an outgroup based on Voelker (2002). A tissue sample of *M. maderaspatensis* was loaned to us by American Museum of Natural History (GenBank numbers AF526470, AY682721).

Morphological analysis

Study skins of 93 males from 24 populations (housed at the Burke Museum (WA), Bell Museum (MN), or Zoological Museum (Moscow, Russia)) were scored for 12 plumage characters (Appendix 1) that have been used to define subspecies (Dement'ev and Gladkov 1954, Cramp 1988): (1) forehead color, (2) crown color, (3) nape color, (4) back color, (5) color of sides of neck, (6) color of ear-coverts, (7) cheek color, (8) chin color, (9) throat color, (10) black stripe through the eye, (11) amount of white on the wing, and (12) depth of black on chest (measured in mm from study skins). Characters 1–9 were scored as white, gray or black, and character 10 as presence or absence. Character 11 was defined as an area of white on the wing divided by the total wing area and was scored from photographs of spread wings using program Scion Image for Windows (available online from Scion corporation at http://www.scioncorp.com/). Three non-overlapping states were assigned to character

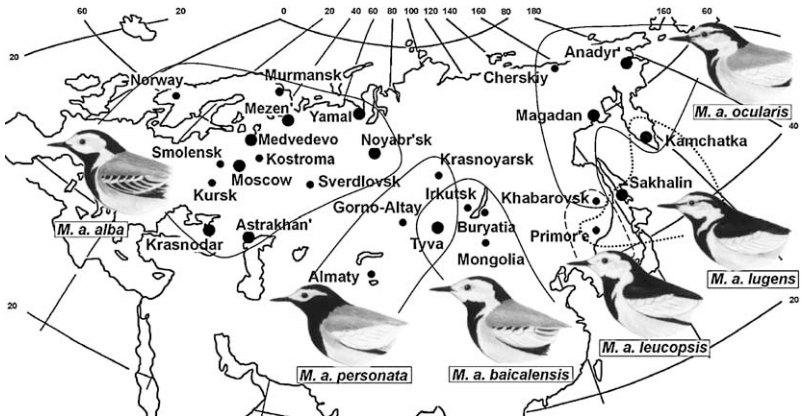

Fig. 1. General location of collecting sites and phenotypic variation of collected birds. Large circles indicate sample sizes of 10 or more individuals. Subspecies were determined following Dement'ev and Gladkov (1954). Intermediate phenotypes are not shown. Subspecies distribution differs from Alström and Mild (2003) and Stepanyan (2003) because not all sympatric subspecies were sampled at each locality. *M. a. dukhunensis* (from Krasnodar, Astrakhan', Sverdlovsk and Noyabr'sk) was pooled with *M. a. alba*. Of the two birds collected at Krasnoyarsk one was *M. a. personata* and the other was either *M. a. alba* or *M. a. baicalensis* (Table 1).

11: white area less than 25% of wing (all subspecies except *M. a. lugens* and its hybrids with *M. a. ocularis*), white area 25–50% of wing (*M. a. lugens* and *M. a. ocularis* hybrids), and white area 50–100% of wing (*M. a. lugens*). Characters 1–11 were used in a maximum parsimony analysis of unique phenotypes (with *M. maderaspatensis* as an outgroup). Character 12 (length of black bib on the chest) was omitted from analysis because discrete states could not be assigned to it. Simple linear regression of values for character 12 on geographical coordinates was performed. Character 12 presumably depended on the way the study skin was prepared. Only breeding males were used for morphological analysis because subspecies identification of females is difficult in some individuals (Cramp 1988). *M. a. dukhunensis* occupies the south-eastern European part of Russia and west-central Asia (Cramp 1988). It differs from *M. a. alba* by wider white borders on the wing coverts, white tertial edges, and in general has more white on the wing (Stepanyan 2003). However, the extent of white area on the wing overlaps considerably between *M. a. alba* and *M. a. dukhunensis*. In this paper we refer to both subspecies as *M. a. alba*. Thus, our sampling included six subspecies (Fig. 1, Table 1).

Molecular lab methods

Isolation and purification of the DNA was performed using phenol-chloroform protocol (Hillis et al. 1996) or QIAamp Tissue Kit (QIAGEN, Valencia, California). Polymerase Chain Reaction (PCR) (Saiki et al. 1988) with Perkin-Elmer PCR reagents was used to amplify two mitochondrial gene regions. Primers L5215 (Hackett 1996) and H1064 (Drovetski et al. 2004) were used to amplify the complete NADH dehydrogenase subunit 2 (ND2) gene. PCR started with 2.5 min at 95°C, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min, and ended with an extension of 10 min at 72°C. Primers LCR4 and H1248 (Tarr 1995) were used to amplify the 3′-end part of the Control Region (CR). The PCRs for CR were performed with 34 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C with final extension for 10 min at 72°C. Qiaquick PCR Purification Kit (QIAGEN) was used to clean PCR products. Then either standard Sephadex columns were used to clean sequencing reactions, which were sequenced on ABI 310 automated sequencer using Dideoxy Terminator Kit Protocol, or cleaned PCR fragments were directly sequenced on ABI 3700 automated sequencer using BigDye chemistry (Applied Biosystems). Amplification primers and primers L347 (Drovetski et al. 2004) and H5578 (Hackett 1996) were used for sequencing of 1041 base pairs (bp) of ND2. Primers LCR4 (Tarr 1995), LCON2 (Zink et al. 1998), 2LCRU (5′-GATGCACTTT-GNCCNCATTC-3′, designed by R. Blackwell-Rago) and 2HCRU (5′-GAATGNGGNCAAAGTGCATC-3′, designed by R. Blackwell-Rago) were used to obtain 436 bp of control region.

Data analysis

Sequences were aligned and edited in Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan). Sequence and locality data have been deposited in GenBank under accession numbers AY681496-AY681963. Mitochondrial origin of sequenced DNA fragments was supported by the absence of stop-codons in ND2 and the existence of a large number of haplotypes (72 for ND2 and 23 for CR alone), which are inconsistent with nuclear copies (Zhang and Hewitt 1996).

PAUP* (Swofford 2000) was used to generate maximum parsimony trees from equally weighted characters, then strict and 50% majority rule consensus trees were constructed. Maximum likelihood (ML) phylogenetic

Table 1. Genetic characteristics of 26 geographic samples of *Motacilla alba* (Fig. 1) as calculated by Arlequin with 0% missing data allowed. Nine individuals from Irkutsk and two from Buryatia were pooled. Subspecies were determined following Dement'ev and Gladkov (1954). N/s- not significant (mismatch distribution is not different from sudden expansion model), $P > 0.05$; *-$P < 0.05$, unim- unimodal, bimod- bimodal distribution, no test- Arlequin failed to fit the model of sudden expansion.

Collecting locality	°North latitude/ °East longitude	Subspecies (both sexes)	Sample size	No. of ind. with most common haplotype	No. of polymorphic sites	No. of haplotypes	Haplotype diversity	Nucleotide diversity $\times 10^4$	Tau	Theta 0	Theta 1	Mismatch pattern	Fu's Fs	Tajima's D
Norway	61/10	*alba*	1	0	–	1	–	–	–	–	–	–	–	–
Kursk	51/34	*alba*	1	0	–	1	–	–	–	–	–	–	–	–
Smolensk	54/34	*alba*	1	1	–	1	–	–	–	–	–	–	–	–
Murmansk	66/34	*alba*	4	2	2	3	0.83	6.8	1.28	0	2645	–	n/s	–
Krasnodar	44/37	*alba (dukhunensis)*	24	2	28	15	0.95	42	1.37	3.88	890	bimod n/s	n/s	n/s
Moscow	55/37	*alba*	31	19	13	11	0.63	7.2	1.27	0	3.59	unim n/s	−8.05*	−2.2*
Medvedevo	60/38	*alba*	16	9	6	7	0.69	5.8	1.09	0	2420	unim n/s	−4.72*	−1.8*
Kostroma	58/41	*alba*	2	0	0	1	0	0	–	–	–	–	–	–
Mezen'	65/44	*alba*	13	4	12	10	0.92	14.6	no est.	no est.	no est.	unim no test	−6.82*	−1.8*
Astrakhan'	46/47	*alba (dukhunensis)*	10	5	5	5	0.76	8.6	no est.	no est.	no est.	unim no test	−1.67*	
Sverdlovsk	56/58	*alba (dukhunensis)*	5	0	2	3	0.7	6.8	1.29	0	1181	–	n/s	n/s
Yamal	68/68	*alba (dukhunensis)*	10	6	5	5	0.67	6.8	1.1	0	1048	unim n/s	−2.26*	−1.7*
Noyabr'sk	63/74	*alba (dukhunensis)*	10	7	3	4	0.53	4	0.76	0	831	unim n/s	−1.96*	−1.6*
Almaty	42/75	*personata*	4	0	10	4	1	33.8	2.24	3.67	3978	–	n/s	n/s
Gorno-Altay	51/85	*personata*	2	1	1	2	1	6.8	–	–	–	–	–	–
Tyva	50/93	*personata, baicalensis,* hybrids[1]	16	12	6	5	0.45	5	2.3	0	0.88	bimod n/s	−2.17*	−2.0*
Krasnoyarsk	57/97	*personata, alba* or *baicalensis*[2]	2	0	0	1	0	0	–	–	–	–	–	–
Irkutsk	54/104	*baicalensis*	9	6	5	5	0.71	7.1	1.17	0	1983	unim n/s	−1.93*	n/s
Buryatia	52/106		2											
Mongolia	47/112	*baicalensis*	4	2	4	3	0.83	13.5	3.4	0	5.3	–	n/s	n/s
Primor'e	44/132	*leucopsis, lugens*[3]	9	3	15	6	0.89	48.9	13.3	0	16	bimod n/s	n/s	n/s
Khabarovsk	51/136	*leucopsis, ocularis*[4]	4	3	1	2	0.5	3.4	0.77	0	1045	–	n/s	n/s
Sakhalin	46/141	*lugens*	12	6	5	6	0.76	8.2	1.33	0	1892	unim n/s	−2.74*	n/s
Magadan	59/150	*ocularis*	13	6	8	7	0.79	9.2	no est.	no est.	no est.	unim no test	−3.59*	−1.83*
Kamchatka	52/157	*lugens, ocularis,* hybrids[5]	11	5	7	6	0.8	11	1.78	0	15	unim n/s	−2.04*	n/s
Anadyr'	64/177	*ocularis*	15	6	11	9	0.85	12.1	1.98	0	1543	unim n/s	−5.0*	−1.81*
Cherskiy	69/158	*ocularis*	1	0	–	1	–	–	–	–	–	–	–	–

[1]One breeding male from Tyva resembled *M. a. personata*, but had the white malar stripe characteristic of winter plumage of *M. a. personata* (Fig. 2A).
[2]From two birds collected in Krasnoyarsk, one was a female *M. a. personata*. Only a skeleton and a wing were preserved from the other bird, this wing differed from a typical wing of *M. a. personata*, so this bird might belong to either *M. a. alba (M. a. dukhunensis)* or *M. a. baicalensis*.
[3]In Primor'e, two individuals of *M. a. lugens* were collected on the shore of Sea of Japan, and seven *M. a. leucopsis* from more inland areas.
[4]There were three individuals of *M. a. leucopsis* and one *M. a. ocularis* collected in Khabarovsk.
[5]Most individuals from Kamchatka possessed intermediate phenotypes between *M. a. lugens* and *M. a. ocularis* with varying amounts of black and gray feathers on the back, a mixture of black and white feathers on the chin, and a white wing patch of various sizes (Figs. 2D and E).

analyses were performed using PAUP*. ML model and parameters were determined by the Hierarchical Likelihood Ratio Test (hLRT) in Modeltest 3.06 (Posada and Crandall 1998).

A likelihood ratio (LR) test was performed on all sequences to evaluate whether the sequences have been evolving in a clocklike manner. Scores from ML trees with and without a molecular clock enforced (Felsenstein 1981) were compared and the LR was calculated as $2(\ln L_{clock} - \ln L_{no\ clock})$ under the assumption that the LR was χ^2 distributed with degrees of freedom (df) equal to the number of taxa minus two (Nei and Kumar 2000).

Arlequin software (Schneider et al. 2000) was used to compute the number of haplotypes in population, nucleotide diversity (π), haplotype diversity (h), number of polymorphic sites (S) and pairwise population Φ_{st}. There were no gaps in sequences, sites with missing data were omitted from analysis. We regressed values of nucleotide diversity (π) against latitude and longitude expecting smaller values to be observed on the leading edge of population expansion (Hewitt, 2000). We also used Arlequin to perform Tajima's D (Tajima, 1996) and Fu's Fs (1997) tests of selective neutrality, analysis of molecular variance (AMOVA; Excoffier et al. 1992), and Mantel's (1967) test of pairwise Φ_{st} values vs. geographic distances. For among-population comparisons we used only populations with sample sizes of four or more individuals. Arlequin was also used to compute mismatch distributions, time since population expansion τ, and effective population sizes before (θ_0) and after (θ_1) expansion for localities with sample sizes greater than 10 individuals. To test the empirical mismatch distribution against a model of sudden expansion we used the generalized non-linear least-squares approach (Schneider and Excoffier 1999). MEGA version 2.1 (Kumar et al. 2001) was used to construct a Minimum Evolution tree from population average uncorrected pairwise sequence differences corrected for within population variability.

Results

Analysis of morphological variation

Characters 1, 2, and 3 did not vary across the subspecies: all of the males in our sample had white foreheads and black crowns and napes. Although the white patch on the wing of *M. a. personata* differed visually from the two white wing-bars on the wing of *M. a. alba*, most subspecies (except *M. a. lugens*) had overlapping ranges of values of white area on the wing that did not allow discrimination between all subspecies. There were slight significant trends for northern ($R^2 = 0.1$) and western ($R^2 = 0.32$) birds having smaller bibs ($P < 0.05$). Bib length averaged smaller in *M. a. alba* than in other subspecies ($P < 0.05$). Phylogenetic analysis of morphological characters 4–11 (data matrix is presented in Appendix 1) showed subspecies to be well-defined with little variation within groups, although several individuals of mixed phenotype could not be assigned to any subspecies (Fig. 2, Appendix 1). More than one phenotype was sampled from Tyva, Kamchatka, Krasnoyarsk, Khabarovsk and Primor'e (Fig. 1, Table 1, Appendix 1). Koblik et al. (2001) and Tsvetkov et al. (2003) reviewed the phenotypic variation of white wagtails collected from Tyva and Kamchatka.

An exhaustive search using the eight parsimony informative characters that describe the 10 unique phenotypes of *M. alba* yielded a maximum parsimony tree (Fig. 3A; length 13, consistency index (CI) 0.69, rescaled consistency index (RC) 0.53), in which

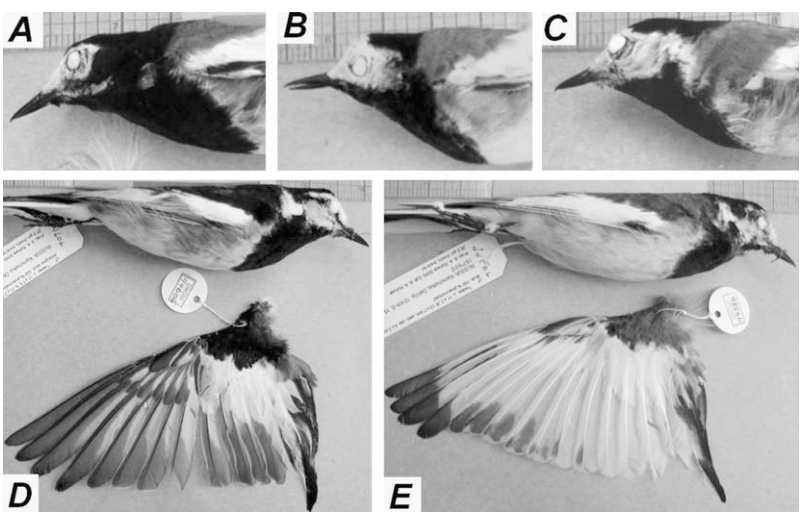

Fig. 2. Intermediate phenotypes of *M. alba* (Fig. 3A): A. *M. a. personata* with white malar stripe from Tyva (148Tyva = UWBM66712). B. Hybrid of *M. a. personata* and *M. a. baicalensis* from Tyva (145Tyva = UWBM66497). C. *M. a. alba* with white chin from Noyabr'sk (44Noya = UWBM56567) (similar phenotypes are also collected from Buryatia (192Bura = UWBM73780) and Mezen' (161Meze = BMNH44770)). D and E. Hybrids of *M. a. ocularis* and *M. a. lugens* from Kamchatka. D (219Kamc = UWBM44606) has black back and intermediate size of white wing patch (same as 216Kamc = UWBM44582, 219Kamc = UWBM44606, 218Kamc = UWBM44587, 224Kamc = UWBM44589), E (222Kamc = UWBM44586) has gray back and large white wing patch.

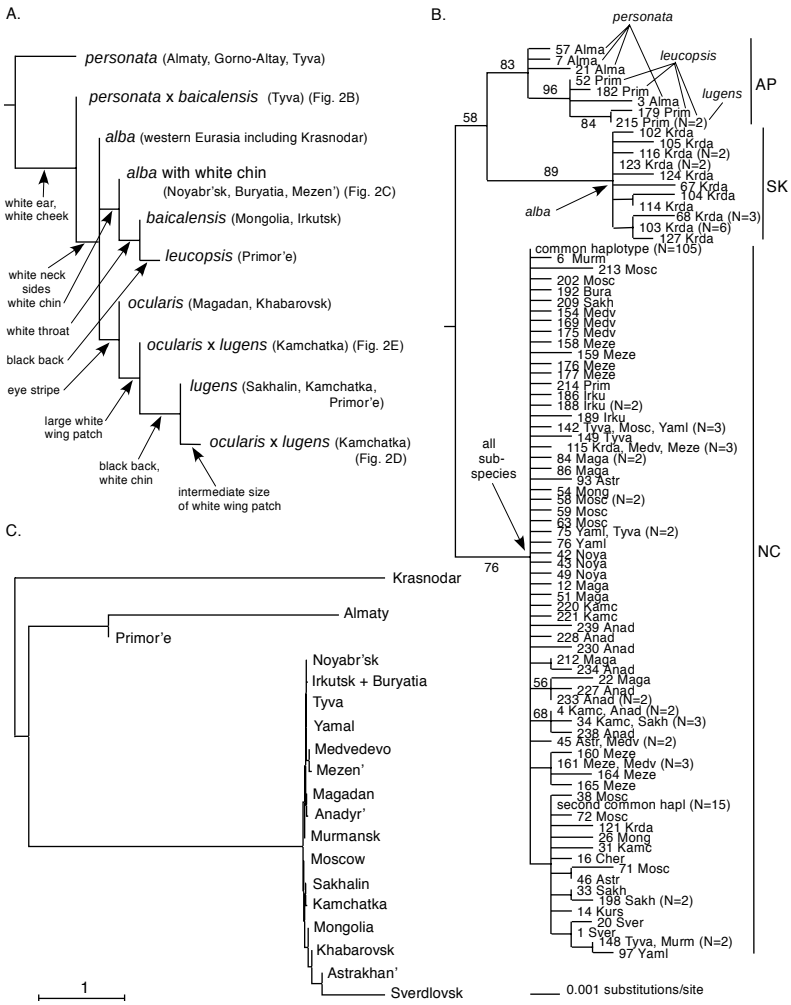

Fig. 3. A. Maximum parsimony tree for 10 unique male phenotypes built from eight phenotypic characters of white wagtails sampled from 24 populations. B. Maximum likelihood tree for 87 unique mtDNA haplotypes. Numbers by branches are bootstrap support from 100 replicates, lab numbers precede locality abbreviations of unique haplotypes, localities where the same haplotype was found are listed, numbers in the brackets are number of individuals sharing the same haplotype. AP- Almaty-Primor'e clade, SK- Southern Krasnodar clade, NC- Northern clade. Abbreviations for localities are: Alma- Almaty, Prim- Primor'e, Krda- Krasnodar, Murm- Murmansk, Mosc- Moscow, Bura- Buryatia, Sakh- Sakhalin, Medv- Medvedevo, Meze- Mezen', Irku- Irkutsk, Yaml- Yamal, Maga- Magadan, Astr- Astrakhan', Mong- Mongolia, Noya- Noyabr'sk, Kamc- Kamchatka, Anad- Anadyr', Cher- Cherskiy, Kurs- Kursk, Sver- Sverdlovsk. *Motacilla maderaspatensis* was used as an outgroup on both trees (A and B). C. Minimum evolution population tree constructed from population pairwise uncorrected sequence differences corrected for within-population differentiation.

M. a. personata was a sister group to the rest of the phenotypes, *M. a. ocularis*, *M. a. lugens* and their hybrids were grouped together, and *M. a. baicalensis* was grouped with *M. a. leucopsis*. According to the morphological tree (Fig. 3A) white chin and black back color evolved twice in two groups of subspecies (one with and one without a black eye stripe).

Molecular phylogenetic analysis

After deleting sites with missing data, only 1468 out of 1477 sequenced nucleotides could be used. ND2 and CR sequences were combined because they are part of the same linkage group. For the 232 individuals sequenced there were 101 polymorphic sites (ten transversions) resulting in 87 haplotypes, excluding the outgroup. Maximum divergence between any pair of sequences of *M. alba* was 1.3%, and divergence between *M. alba* and *M. maderaspatensis* was 3%–3.6%.

The most common haplotype was found in all populations except seven (Norway, Kursk, Kostroma, Sverdlovsk, Almaty, Krasnoyarsk and Cherskiy). This haplotype was shared by 105 individuals (45%; Table 1). The second common haplotype was shared by 15 individuals from nine populations (Norway, Moscow, Kostroma, Astrakhan', Sverdlovsk, Gorno-Altay, Krasnoyarsk, Khabarovsk and Sakhalin; Table 1).

Hierarchical likelihood criteria implemented in Modeltest 3.06 suggested TrN+G (Tamura and Nei 1993) as the most probable model of sequence evolution. Maximum likelihood (ML) analysis with parameters estimated from the data yielded two trees, with three major clades in common (Fig. 3B). One clade included 20 out of the 24 individuals sampled from the Krasnodar region near the Caucasus mountains. The second clade, which was sister to the first, included all four individuals sampled from Almaty and five of the nine birds from Primor'e. The third clade comprised the rest of the individuals including the two most common haplotypes. We refer to the first clade as "southern Krasnodar"

(SK), to the second clade as "southern Almaty-Primor'e" (AP) and to the third clade as "northern clade" (NC). We also refer to the Krasnodar, Almaty and Primor'e localities together as "southern populations" and to the rest of localities as "northern". Four individuals from Krasnodar and four from Primor'e possessed haplotypes that belonged to the northern clade. No haplotypes from the northern clade were found in Almaty, suggesting little or no gene flow between this locality and northern parts of the species' range. The molecular clock hypothesis was not rejected ($-\ln L$ without molecular clock enforced $=3122.53$, $-\ln L$ with molecular clock $=3082.97$; df $=85$, $P=0.99$).

For the MP analysis, the 41 parsimony informative characters resulted in more than 5,000 equally parsimonious trees (length $=170$, CI $=0.82$, RC $=0.71$) whose strict consensus (not shown) recovered same three ML clades as on the ML tree (Fig. 3B). Again, three individuals from Almaty were basal to the rest of Almaty-Primor'e clade. A haplotype from Magadan was basal to the other northern haplotypes (NC clade) in 86 percent of trees.

The phenotypic and haplotypic trees (Fig. 3A, B) were incongruent. Subspecies did not correspond to monophyletic groups of haplotypes.

Genetic variability

Overall nucleotide diversity was 0.0026 for all samples. Nucleotide diversity (π; Table 1) was high for southern samples: Primor'e (0.0049), Krasnodar (0.0042), and Almaty (0.0034). For northern localities π was low and ranged from 0.0003 in Khabarovsk to 0.0015 in Mezen' (average $=0.0009$). Regression of π on sample size showed independence of these variables ($P=0.77$).

Genetic diversity and gene flow

Overall haplotype diversity was 0.79 and ranged from 0.45 in Tyva to 1 in Almaty (Table 1). AMOVA showed a high level of population differentiation; 50.8% of molecular variance was due to comparisons among geographical localities ($P<0.05$). When samples were divided into three groups (Krasnodar, Almaty with Primor'e and all northern samples pooled together), 75.8% of variance distributed among groups ($\Phi_{ct}=0.76$, $P<0.05$) and 23.1% within populations. An AMOVA based on subspecies failed to explain genetic variation ($\Phi_{ct}=-0.11$, $P=0.66$). Overall Φ_{st} for only northern populations was low (0.07, $P<0.05$) with 93% of the variance being accounted for by differences among individuals within populations.

Significant pairwise Φ_{st}-values (Table 2) between geographic localities ranged from 0.02 (Tyva-Magadan) to 0.85 (Tyva-Almaty). All Φ_{st}-values involving Krasnodar were large and significant (ranging from 0.5 to 0.71). Comparing Krasnodar with neighboring Astrakhan' yielded an Φ_{st} of 0.64, showing a high degree of population differentiation with restricted or no gene flow between these samples. High pairwise Φ_{st} values were observed for the Almaty and Primor'e populations, although values for Primor'e-Almaty, Primor'e-Mongolia and Primor'e-Khabarovsk comparisons were not significant, indicating possible gene flow to and from Primor'e. The Primor'e-Murmansk comparison also did not yield a significant Φ_{st} value, but given the large geographic distance between these localities, this likely resulted from sharing the common haplotype rather than from gene flow. All pairwise Φ_{st} values for the Sverdlovsk population were also large and significant which implies a history of isolation of the Sverdlovsk population from other populations we sampled. The remaining pairwise Φ_{st} values either were not significant or were less than 0.1, indicating generally high levels of gene flow.

A minimum evolution tree for populations (Fig. 3C) displayed the same pattern as the ML and MP trees: northern populations were grouped together (with Sverdlovsk being on a long branch), and Krasnodar was a sister group to the clade containing the Primor'e and Almaty populations (with Almaty being on a long branch).

Mantel's test was performed only for northern populations because it was obvious from the phylogenetic analysis (Fig. 3B) that some other cause than isolation-by-distance was responsible for the genetic structure observed across all localities. No isolation-by distance effect was detected for northern populations ($R^2=0.006$, $P=0.74$).

Regression of nucleotide diversity against latitude yielded an R^2 of 0.30 ($P<0.05$, Fig. 4). However, statistical significance was due to high values of π for the three southern populations: Krasnodar, Almaty and Primor'e. Considering the fact that the northern samples were all more closely related to each other than to any of the southern samples, the test might not be valid due to violation of independence. When only northern localities were analyzed, there was no association between latitude and nucleotide diversity. Regression of π against longitude for all samples, and northern sites only, did not result in a significant R^2. Therefore, we found no signature of leading edge expansion.

Population expansion

Pairwise differences within each northern population were distributed in accordance with the model of sudden population expansion (Fig. 5, Table 1) and had unimodal distributions (with the exception of Tyva). Both Krasnodar and Primor'e included individuals from two different clades and, therefore, had bimodal mismatch

Table 2. Population pairwise Φ_{st}'s for comparisons between populations with sample sizes of four or more individuals. Values are presented only if significant at 0.05 level, values larger than 0.3 are in bold. Nine individuals from Irkutsk and two from Buryatia were pooled.

	Mur	Krd	Mos	Med	Mez	Ast	Sve	Yam	Noy	Alm	Tyv	IrBu	Mon	Prim	Kha	Sak	Mag	Kam
Murmansk	–																	
Krasnodar	**0.59**	–																
Moscow		**0.71**	–															
Medvedevo		**0.67**		–														
Mezen'		**0.64**	0.07		–													
Astrakhan'		**0.64**		0.07	0.07	–												
Sverdlovsk	**0.47**	**0.63**	**0.44**	**0.56**	**0.39**	**0.32**	–											
Yamal		**0.64**					**0.5**	–										
Noyabr'sk		**0.64**					**0.61**		–									
Almaty	**0.72**	**0.53**	**0.84**	**0.83**	**0.74**	**0.78**	**0.75**	**0.8**	**0.82**	–								
Tyva		**0.68**			0.07	0.08	**0.6**			**0.85**	–							
Irkutsk + Buryatia		**0.64**		0.03		0.07	**0.52**			**0.8**	0.03	–						
Mongolia		**0.58**					**0.31**			**0.68**			–					
Primor'e		**0.5**	**0.56**	**0.5**	**0.42**	**0.42**	**0.42**	**0.42**	**0.44**		**0.51**	**0.44**		–				
Khabarovsk		**0.59**					**0.43**			**0.73**					–			
Sakhalin		**0.65**		0.07	0.08		**0.41**			**0.79**	0.07	0.06		**0.44**		–		
Magadan		**0.65**	0.03		0.05	0.06	**0.45**			**0.79**	0.02			**0.44**		0.05	–	
Kamchatka		**0.63**	0.06	0.07	0.07		**0.39**			**0.76**	0.08			**0.42**				–
Anadyr'		**0.65**	0.05	0.04	0.06	0.06	**0.40**			**0.76**	0.03			**0.44**				

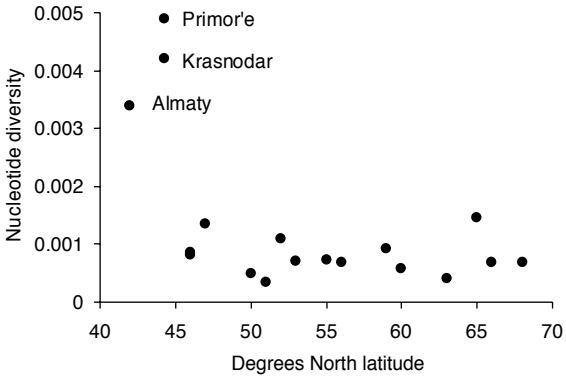

Fig. 4. Plot of nucleotide diversity versus latitude for all localities with sample sizes of four or more individuals. Latitude explains 30% of the variance in nucleotide diversity when all samples are analyzed.

distributions (Fig. 6), but these localities still did not differ from a model of sudden expansion using the bootstrap test of Schneider and Excoffier (1999). The mismatch distribution for the southern Krasnodar (SK clade) haplotypes (Fig. 6) was unimodal and also did not differ significantly from the sudden expansion model. Although southern haplotypes from Krasnodar (SK clade) did display the signature of past population growth, this expansion was older than those in the northern clade (NC), as can be judged from the right shifted mode for the Krasnodar mismatch distribution (Figs. 5 and 6). Estimates of tau (τ) for northern localities ranged from 0.76 in Noyabr'sk to 3.4 in Khabarovsk (Table 1), but were not structured geographically.

Tests of selective neutrality

Fu's Fs values (Table 1) were not significantly for Krasnodar, Almaty, Primor'e, Murmansk, Sverdlovsk, Mongolia and Khabarovsk. For the rest of northern localities Fu's Fs were significantly negative ($P < 0.05$). This result suggests either deviations from neutral evolution in stable populations or population growth.

The values of Tajima's D (Table 1) were significantly negative ($P < 0.05$) for Moscow, Medvedevo, Mezen', Yamal, Noyabr'sk, Tyva, Magadan and Anadyr'. Rejection of neutrality is also consistent with a recent bottleneck and lack of demographical equilibrium.

Discussion

Plumage evolution

Our phylogenetic tree constructed from morphological characters (Fig. 3A) is mostly consistent with the groups suggested by Cramp (1988), but not in the relationships among groups, because *M. a. lugens* and *M. a. ocularis* were not most closely related to *M. a. personata*. Our tree was not consistent with the two groups of subspecies as suggested by Ödeen and Alström (2001), because *M. a. personata* had more similarities with the outgroup, the white-browed wagtail, than with *M. a. leucopsis*, and *M. a. leucopsis* was most closely related to *M. a. baicalensis*.

Several potentially convergent evolutionary patterns emerged from our phenotypic tree (Fig. 3A): black back color evolves in *M. a. lugens* and in *M. a. leucopsis*, making these subspecies distinguishable from *M. a. ocularis* and *M. a. baicalensis* respectively. Two additional taxa that we did not sample develop black backs: *M. a. yarrellii* from Britain and Ireland, which looks much like nominate *M. a. alba* except for the black back, and *M. a. alboides* from Himalayas, which differs from *M. a. personata* of central Asia only by its black back. Another character state evolving more than once is white

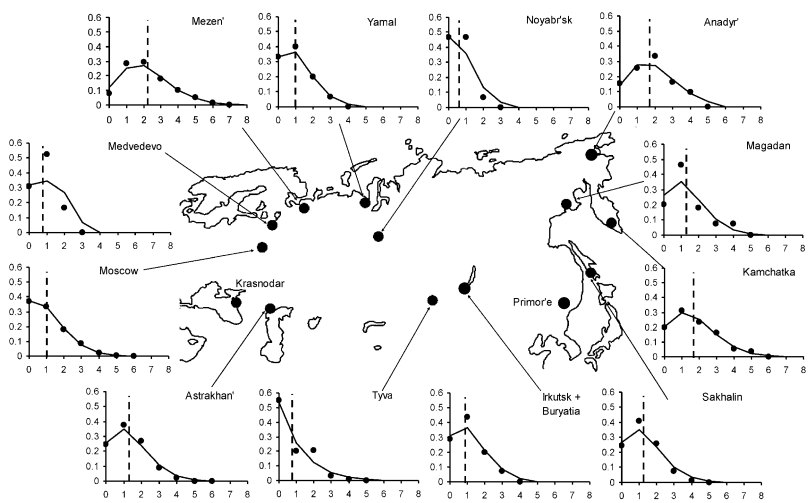

Fig. 5. Mismatch distributions for localities with sample sizes of ten or more individuals for northern localities. X-axis- number of pairwise differences between sequences; Y – axis – frequency; black dots indicate observed values, lines show model distribution as calculated by Arlequin (Schneider et al. 2000), vertical dashed line indicates the mean of the distribution.

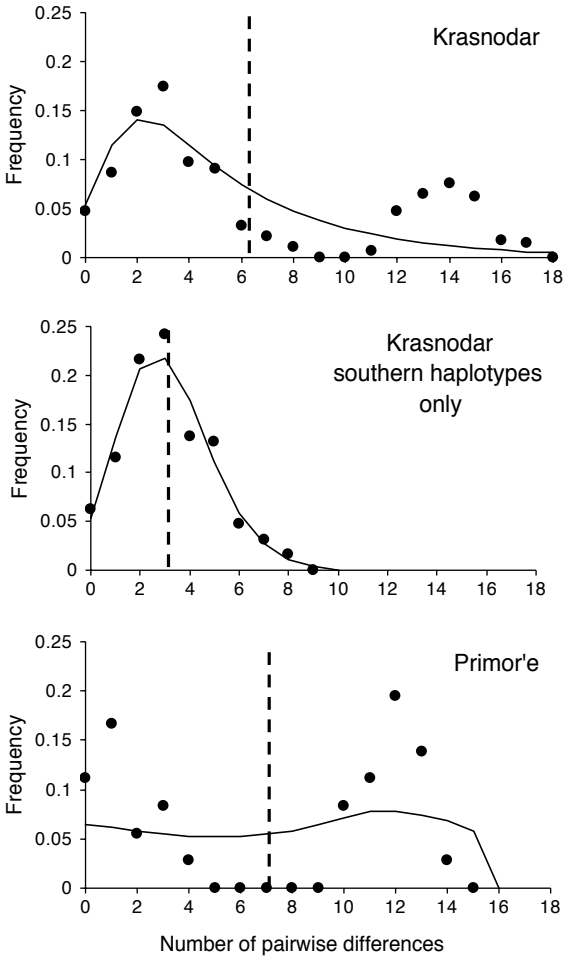

Fig. 6. Mismatch distributions for all Krasnodar individuals, only southern Krasnodar (SK clade) haplotypes and all individuals from Primor'e. Black dots indicate observed values, line- model distribution as calculated by Arlequin (Schneider et al. 2000), dashed line indicates the mean of the distribution.

chin color (Fig. 3A). Interestingly, both back and chin color change during the prebasic molt: all black-throated subspecies develop white chins and throats in their winter plumage and some of black-backed birds become gray-backed in winter (Badyaev et al. 1996, Alström and Mild 2003). It is possible that summer plumage characters primarily function in male-male competition and are of little importance to female mate choice. The more this is true, the more likely will be the occurrence of mixed pairings and the development of hybrid zones where different subspecies make contact. Such interbreeding facilitates high gene flow between populations. Given that up to six subspecies can be present on the same wintering grounds in India or southeast Asia (Alström and Mild 2003), molting into virtually indistinguishable phenotypes might be adaptive to winter survival in flocks, because this prevents intrasexual aggression (Røskaft and Rohwer 1987).

Incongruence of morphological and molecular trees

Our analysis of mtDNA variation does not support subspecies relationships suggested by Cramp (1988), or Ödeen and Alström (2001), because none of the subspecies studied by us formed a clade. Among avian species, distinctive plumages are almost always accompanied by fixed differences in mtDNA, but the situation is different at the population and subspecies level. For example, in yellow wagtails *Motacilla flava* many birds of very different appearances possessed the same mtDNA sequences, whereas in several cases similarly looking birds did not share a recent common ancestor (Pavlova et al. 2003). Although plumages of birds can reflect the evolutionary history of populations (Alström and Mild 2003), mtDNA evidence often does not support morphologically defined subspecies as evolutionary units (Ball and Avise 1992, Zink 2004). Thus, mtDNA and morphological patterns can vary independently, as observed here for white wagtails.

There were three clades on the mtDNA gene tree of white wagtails. The northern clade (NC) included individuals from all subspecies, the southern Krasnodar (SK) clade included only birds belonging to *M. a. alba* subspecies, and the southern Almaty-Primor'e (AP) clade included three phenotypes: *M. a. personata*, *M. a. leucopsis* and *M. a. lugens* (Fig. 3B). In some localities individuals from two non-sister clades shared the same phenotypes. In Krasnodar, the birds from southern (SK) mtDNA clade do not share a most recent common ancestor with the individuals belonging to northern (NC) clade, although phenotypically they all belong to *M. a. alba* subspecies, as do most of the birds from western Eurasia (Fig. 7A). *M. a. personata* was also represented by individuals from two different clades. Therefore, with respect to mtDNA, individuals with *M. a. personata* phenotype from Almaty that belonged to Almaty-Primor'e (AP) clade in mtDNA tree were not closest relatives to *M. a. personata* from Gorno-Altay and Tyva, which possessed haplotypes from northern (NC) clade (Fig. 7B).

There are several alternative explanations for the observed distribution of haplotypes and phenotypes. For example, phenotypically similar members of both clades could have retained ancestral plumage. However, this could only explain phenotype-haplotype distribution of only one of the subspecies. Alternatively, sexual or natural selection could favor a particular phenotype in a region irrespective of the mitochondrial background of individuals. Lastly, sex-biased dispersal, where females are more philopatric than males, could have prevented haplotypes from mixing while facilitating plumage similarity. However, none of these interpretations explain the complicated phenotype-haplotype distributions in Primor'e. Both subspecies represented in our sample from Primor'e had individuals belonging to two mtDNA clades (Fig. 7C). Three individuals with

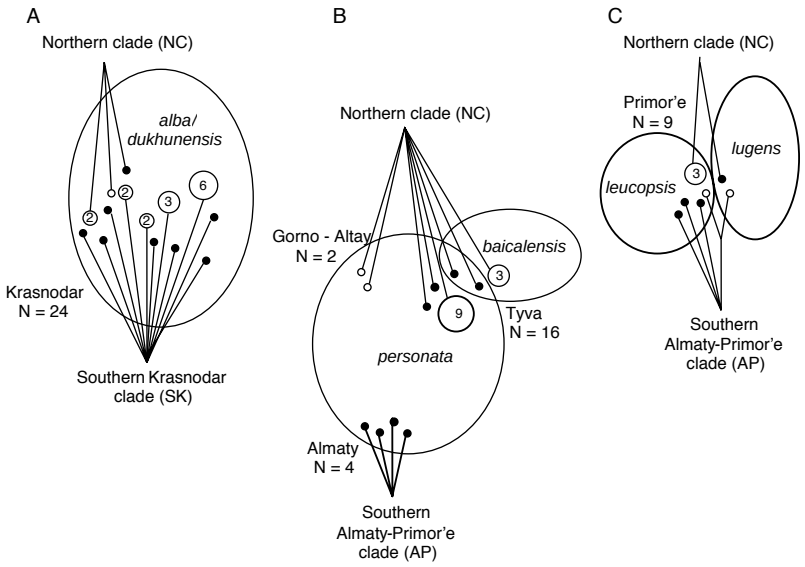

Fig. 7. Haplotype and phenotype (both sexes) distributions of white wagtails at several localities. A. Krasnodar. B. Tyva, Almaty and Gorno-Altay. C. Primor'e. Sample sizes are given under locality names. Black circles indicate unique haplotypes, small white circles indicate haplotypes shared among different localities or subspecies, number inside white circles is number of individuals from the same locality sharing same haplotype; large ovals indicate subspecies, area of their overlap indicate hybrid phenotypes.

M. a. leucopsis phenotype shared the most common haplotype of the northern (NC) clade, whereas the remaining four *M. a. leucopsis* belonged to the southern Almaty-Primor'e (AP) clade. Two *M. a. lugens* from Primor'e also belonged to different clades. It is possible that introgression of northern haplotypes to Krasnodar and Primor'e occurs via females that were hatched in the north but stopped "short" to breed in more southern localities. If sexual or natural selection drives evolution of phenotypes, then it must have acted much faster than mtDNA lineage sorting, because none of the subspecies are monophyletic on the mtDNA tree.

The absence of congruence between morphological and mtDNA variation can be explained by the different time frames of evolutionary history that are recovered with these data sets. It takes approximately $2Ne_f$ generations (Ne_f is inbreeding effective female population size) for mtDNA to reach the stage of reciprocal monophyly, whereas the evolution of morphological characters can take several orders of magnitude less time, because these characters are likely polygenic and under the relentless force of sexual or natural selection (Rohwer 1982, West-Eberhard 1983, Rohwer and Røskaft 1989). Complicated dispersal events at different times in species' evolution can obscure the effects of earlier isolating events. Field experiments establishing whether rapid color evolution is driven and maintained by male-male competition, by female choice, or by other forces are needed.

Phylogeography and Pleistocene history of populations

Diversity in mtDNA was low across the continent with 38% of haplotypes being found in single individuals and 45% of the individuals from distant populations sharing the same haplotype. The number of unique haplotypes is low compared to yellow wagtails, where for sequences of about 1000 bp, 77% of haplotypes from the western clade, 54% from the northeastern clade and 85% from the southeastern clade were unique (Pavlova et al. 2003). Insignificant values from Mantel's test and the presence of two widespread haplotypes suggest a history of recent gene flow between northern sampling sites for white wagtail. Thus, although congeneric, and both consisting of three haplotype groups, white and yellow wagtails experienced different recent histories.

Observed pairwise Φ_{st}–values for the Sverdlovsk population were surprisingly high given the absence of geographical barriers. These high Φ_{st}–values may be an artifact of small sample size ($N = 5$), because the most common haplotype, which represented a large fraction of individuals in most northern populations, was not sampled in Sverdlovsk. Therefore, more samples from Sverdlovsk are needed before definitive conclusion about population isolation can be made.

Deep phylogeographic splits are traditionally interpreted as the result of a long-term isolation. When the geographic distributions of the clades overlap, as with white wagtail, at least two alternative interpretations are possible: historical isolation with recent secondary contact, or incomplete lineage sorting. It is unlikely that the mixture of southern and northern haplotypes in Krasnodar (individuals from SK and NC clades) and Primor'e (AP and NC clades) is the result of incomplete lineage sorting, because the three clades are geographically structured with Krasnodar and Primor'e being on the borders of the clade distributions. Our Krasnodar sample includes birds collected at six "localities". The four Krasnodar birds with northern haplotypes (from NC) were all collected at sea level near the northeastern

shore of the Black sea and the eastern shore of Azov sea (two localities from the northwestern part of Krasnodar region). Whereas some individuals with southern haplotypes (SK clade) were also found in these localities, the other birds with southern haplotypes (SK clade) were collected at higher elevations on the slopes of Caucasus mountains (four localities). No haplotypes from the Krasnodar (SK) clade were found in Astrakhan', even though these populations are less than 900 km apart. According to the haplotype tree and pairwise Φ_{st}-values, there seems to be no northward gene flow from Almaty to Tyva or Gorno-Altay. Unfortunately our sample sizes from Almaty and Gorno-Altay were too small to allow strong conclusions and larger samples from these areas could reveal haplotypes that are shared between these populations. The occurrence of the most common haplotype in most northern localities, in Krasnodar and Primor'e suggests movement of genes from northern to southern localities. Therefore, we believe that the best historical scenario for white wagtail is historical isolation of the southern parts of the range (Krasnodar, Almaty and Primor'e), with Primor'e and Krasnodar being the zones of secondary contact between two clades.

Irwin (2002) showed that deep phylogeographic splits may arise without geographic barriers to gene flow. The likelihood of observing such splits increases as the individual dispersal distance and population size increase. Whereas the presence of a geographic barrier (Caucasus) to gene flow between southern Krasnodar (SK) and northern (NC) clades is evident, the cause of the split between Krasnodar (SK) and southeastern localities (Almaty and Primor'e, AP) is not obvious. Although at present there are no apparent geographic barrier between Krasnodar and Almaty, such barrier could have existed in the past. It is also possible that phylogeographic split between two southern clades (SK and AP) is the result of stochastic processes and not of a geographic barrier (Irwin 2002).

In general, a unimodal distribution of pairwise differences between individuals in each population indicates that the population has been growing at some point in the past, whereas bimodal or ragged distributions suggest stable populations (Rogers and Harpending 1992). If recent secondary contact between southern and northern haplotypes in Primor'e and Krasnodar is the true evolutionary scenario, as we believe it is, then the conclusion of population growth is suspect because the sudden expansion model assumes a single closed population. Therefore, estimates of population parameters are unreliable for Krasnodar and Primor'e. We conclude that Krasnodar population (modern southern Krasnodar (SK) haplotypes, excluding immigrant haplotypes), and most of our northern populations, have undergone population expansion, with Krasnodar expansion predating expansions on the north. This is a typical finding in temperate avian population and is consistent with the idea that species that survived Pleistocene climatic changes experienced population expansion as they recolonized northern regions.

No evidence of bottlenecks was detected by Tajima's D test for the two southern clades (SK and AP) and southern populations from the northern clade (NC): Astrakhan', Sverdlovsk, Baikal (Irkutsk and Buryatia), Mongolia, Sakhalin and Kamchatka. High adaptability of the white wagtail to a range of climates could have allowed relatively large populations to survive the Late Pleistocene, when most of these sample localities would have been covered with steppe-tundra (Adams 2002). Meanwhile, most of Europe was covered by glacial ice, and polar desert was in northern Siberia and the Sayan mountain region (Adams 2002). Therefore, the significantly negative Tajima's D-values (Table 1) for Moscow, Medvedevo, Mezen', Yamal, Noyabr'sk, Tyva, Magadan and Anadyr', suggest that these populations went through bottlenecks. Unimodal mismatch distributions (Fig. 5) reveal that these populations have expanded in size.

At the same time the ancestral Krasnodar population could have survived in large numbers in the warmer climate southeast of the Black Sea, being separated from northern populations by Caucasus glaciation. Our samples from Primor'e come from the zone of Pleistocene grasslands (Adams 2002). Primor'e was isolated from Sakhalin by polar desert at the mouth of the Amur river, which could have prevented contact between northern haplotypes (from NC) and haplotypes from Almaty-Primor'e (AP) clade. Meanwhile the Almaty population is situated in the zone of polar desert, so birds likely arrived in this region from more eastern grasslands when the climate became more suitable. Warmer climates of the southeast (contemporary eastern China) may have allowed wagtails to maintain fairly high effective population sizes, which would explain the higher nucleotide diversity in Almaty-Primor'e (AP) clade.

We hypothesized that ancestors of modern birds with northern haplotypes (NC) might have survived maximum cooling of Late Pleistocene in the steppe-tundra of the Far Eastern part of Eurasia, because more western parts of Siberia were mostly covered with polar desert (Adams 2002). A haplotype from Magadan was basal to the rest of the northern haplotypes on most of MP trees, thus supporting an eastern origin of the northern clade (NC). However, we did not find a westward decrease in nucleotide diversity in northern populations that would be expected if birds from eastern populations (Kamchatka, Sakhalin, Magadan, Anadyr') dispersed westward. In general, nucleotide diversity in the northern (NC) clade was much lower than in southern (SK and AP) clades. We conclude that white wagtail relatively recently colonized the north of its range, but there is no genetic signature of leading edge expansion (in any

direction) to indicate directionality, unlike the case in other studies (Merilä et al. 1997).

Species limits

Although *M. a. lugens* is the only subspecies found on Sakhalin, both *M. a. ocularis* and *M. a. lugens* occur together and hybridize in Kamchatka, exhibiting intermediate values of the characters that are used to distinguish *M. a. lugens* from *M. a. ocularis* (large amount of white on the wing and black back; Koblik et al. 2001, Rohwer et al. 2001). There is no support for classifying *M. a. lugens* as a species regardless of species concept applied because these forms share the same haplotypes and extensively hybridize. There is also no support in mtDNA for recognition of *M. a. personata* as a species.

The mtDNA tree is one of many possible gene trees because all mitochondrial genes are linked and maternally inherited. Thus, the mtDNA tree may or may not reflect the true evolutionary history of populations. There are several ways to interpret the phylogenetic tree of mitochondrial haplotypes for the white wagtail. If one believes that the three clades represent three historically isolated taxa that just recently came into contact, then three species could be recognized. These clades have narrow zones of secondary contact, just north of the Caucasus and in southern Primor'e. However, these three clades have no clear diagnostic morphological differences. Alternatively, on the basis of high recent gene flow between most northern populations and a widespread haplotype that occurs in most populations including Krasnodar and Primor'e, it could be postulated that *M. alba* is a single biological species. Lastly, one might also argue that morphological subspecies should be recognized as recently evolved species if the narrow hybrid zones are stable and maintained by selection against hybrids. Extensive morphological study of hybrid zones would be needed to test the latter interpretation. We suggest that the white wagtail is best considered as a single species, given available data.

Note in proof

Recently we sequenced two individuals of *Motacilla alba alboides* from Vietnam (approximate coordinates 23°N 105°E; courtesy of American Museum of Natural History, GenBank numbers AY681728-AY681729, AY681962-AY681963). Both sequences were unique and belonged to Almaty-Primor'e clade, further supporting the southeastern origin of this clade.

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Appendix 1. Geographic distribution of 10 phenotypes scored from 93 males of white wagtails and phenotypic data matrix used in phylogenetic analysis.

Locality	Sample size	Phenotype	Character matrix[1]	Voucher location[2]	Lab numbers (museum numbers)
Moscow	15	*alba*	10002203	UWBM	63 (61016), 112 (61645), 60 (61008), 62 (61014), 59 (61007), 109 (61613), 25 (61321), 24 (61317), 96 (59518), 37 (49586), 5 (49570), 38 (49602), 39 (49607), 213 (57388), 110 (61631)
Kostroma	1	*alba*	10002203	UWBM	139 (66420)
Murmansk	2	*alba*	10002203	UWBM	6 (49718), 200 (49745)
Kusrk	1	*alba*	10002203	UWBM	14 (49417)
Norway	1	*alba*	10002203	UWBM	89 (58385)

Appendix 1 (*Continued*)

Locality	Sample size	Phenotype	Character matrix[1]	Voucher location[2]	Lab numbers (museum numbers)
Krasnodar	6	*alba*	10002203	UWBM	105 (61456), 124 (64724), 115 (64607), 114 (64606), 104 (61449), 65 (61069)
Almaty	1	*personata*	12222203	UWBM	21 (46419)
Gorno-Altay	1	*personata*	12222203	UWBM	80 (46564)
Tyva	4	*personata* (148 with white malar Fig. 2A)	12222203	UWBM	141 (66313), 143 (66453), 144 (66468), 148 (66712)
	1	*personata* × *baicalensis* (Fig. 2B)	12002203	UWBM	145 (66497)
Mongolia	2	*baicalensis*	10000003	UWBM	26 (59795), no lab number (60036) (no sequence available)
Astrakhan'	5	*alba*	10002203	UWBM	40 (56447), 94 (56866), 91 (56835), 46 (56667), 48 (56669)
Sverdlovsk	5	*alba*	10002203	UWBM	1 (49525), 36 (49555), 20 (49536), 13 (49543), 35 (49554)
Noyabr'sk	4	*alba*	10002203	UWBM	49 (56824), 43 (56551), 29 (56966), 30 (56976)
	1	*alba* with white chin (Fig. 2C)	10000203	UWBM	44 (56567)
Yamal	2	*alba*	10002203	UWBM	100 (59637), 74 (59408)
Khabarovsk	1	*ocularis*	10002213	UWBM	11 (47169)
Sakhalin	6	*lugens*	20000211	UWBM	198 (46991), 210 (47267), 204 (47246), 205 (47243), 197 (46962), 32 (47244)
Kamchatka	1	*lugens*	20000211	UWBM	31 (44605)
	4	*ocularis* × *lugens* (Fig. 2D)	20000212	UWBM	216 (44582), 219 (44606), 218 (44587), 224 (44589)
	1	*ocularis* × *lugens* (Fig. 2E)	10002211	UWBM	222 (44586)
Magadan	3	*ocularis*	10002213	UWBM	82 (44440), 22 (43837), 55 (44099)
Kirov	1	*alba*	10002203	UWBM	223 (74268)
Buryatia	1	*alba* with white chin (Fig. 2C)	10000203	UWBM	192 (73780)
Irkutsk	5	*baicalensis*	10000003	UWBM	191 (73748), 185 (73462), 189 (73658), 183 (73435), 186 (73533)
Medvedevo	7	*alba*	10002203	BMNH	152 (44271), 153 (44296), 154 (44311), 162 (44206), 163 (44234), 172 (44585), 174 (44401)
Mezen'	9	*alba*	10002203	BMNH	164 (44576), 166 (44584), 167 (44585), 176 (44469), 177 (44472), 178 (44473), 158 (44691), 159 (44692), 160 (44697)
	1	*alba* with white chin (Fig. 2C)	10000203	BMNH	161 (44700)
Primor'e	1	*leucopsis*	20000003	MSUZM	182 (R118104)

[1]Character labels: 1-back color, 2- color of sides of neck, 3- color of ear-coverts, 4- cheek color, 5- chin color, 6- throat color, 7- black stripe through the eye, 8- size of white area on the wing. For characters 1-6: 0-white, 1- grey, 2- black; for character 7: 0- absent, 1- present; for character 8: 1- white area 50–100% of wing, 2- white area 25–50% of wing, 3- white area less than 25% of wing.

[2]UWBM- Burke Museum, University of Washington; BMNH- Bell Museum, University of Minnesota; MSUZM- Zoological Museum, Moscow State University.